Kidney International, Vol. 54 (1998), pp. 27–37

Transplantation of developing metanephroi into adult rats

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Background. Transplantation of developing metanephroi into adult hosts has been proposed as a means to augment host renal function.

Methods. We implanted whole metanephroi from embryonic day 15 (E15) rats subcapsularly in kidneys or into the omentum of non-immunosupressed adult rat hosts. At the time of implantation, some host rats underwent unilateral nephrectomy (UNX) or unilateral nephrectomy and partial contralateral renal infarction (1½ NX). E15 metanephroi contained only metanephric blastema, segments of ureteric bud, and primitive nephrons with no glomeruli.

Results. Four to six weeks post-implantation, metanephroi from E15 rats had enlarged, become vascularized, and had formed mature tubules and glomeruli. Ureters of metanephroi transplanted into the omentum were anastomosed to hosts' ureters that remained after UNX. Four weeks following ureteroureterostomy, the contralateral kidney was removed. Inulin clearances of seven metanephroi implanted into UNX hosts averaged 0.11 ± 0.02 μl/min/100 g (2.42 ± 0.70 μl/min/g kidney wt) and the creatinine clearances averaged 0.65 ± 0.18 μl/min/100 g. Metanephroi weighed 71 ± 15 mg (approximately 4% of the contralateral native kidney). The transplanted metanephroi were vascularized by arteries originating from the omentum. Both weights of transplanted metanephroi (145 ± 24 mg) and inulin clearances of transplanted metanephroi (30.1 ± 8.7 μl/min/g kidney weight) were significantly increased in rats that underwent 1½ NX compared to UNX. In contrast, transplantation of developed kidneys resulted in rejection.

Conclusions. Our findings establish that functional chimeric kidneys develop from metanephroi transplanted in adult hosts.

End-stage chronic renal failure afflicts more than 250,000 individuals in the United States alone, most of whom are treated using dialysis, a treatment with considerable morbidity [1], or renal allotransplantation, which is limited by the number of organs available to transplant [2].

A possible solution for the lack of organ availability is the transplantation of developing kidneys (metanephric allografts or xenografts). There are two theoretical reasons why the transplantation of allograft (or xenograft) metanephroi into adult animals might be advantageous relative to the transplantation of kidneys. First, for several days following its formation, the metanephros has no vasculature [3] and therefore contains few or no antigen presenting cells derived from the circulation. Depletion of antigen presenting cells (or passenger lymphocytes) would be expected to render allograft (or xenograft) metanephroi less immunogenic [4]. Second, the transplanted metanephros becomes a chimeric organ in that it is vascularized in part by blood vessels originating from the host. It is unclear if microvessels within the kidney derive from host or donor tissue [5, 6]. Rejection that is initiated by antibodies directed against antigens on endothelial cell surfaces, is circumvented to the extent that the transplanted organ is supplied by host vessels.

The possibility that renal function can be enhanced through the addition of functioning nephrons via transplantation of allograft metanephroi intrarenally or intraabdominally has been explored in several studies [5–9]. The results of these investigations indicate that transplantation of metanephroi into adult hosts is possible, but complicated by graft rejection within a few days, even if donors and hosts are of the same strain [5]. To readdress this issue, we implanted whole metanephroi from Sprague-Dawley rat embryos subcapsularly into kidneys or into the omentum of outbred adult Sprague-Dawley rats. The survival, growth, maturation, vascularization and function of the metanephroi evident post-transplantation indicate that renal organogenesis can occur under these conditions and that a vascularized functional chimeric kidney is the result. To our knowledge, these studies represent first successful transplantation of metanephroi into the omentum of adult hosts and the first demonstration of plasma clearance in developed transplanted metanephroi.

METHODS

Metanephroi were surgically dissected from embryonic day 15 (E15) Sprague-Dawley rat embryos under a dissecting microscope using previously described techniques [10], and implanted within 45 minutes under the capsule of viable renal tissue or in the omentum of anaesthetized six-week-old female Sprague-Dawley (host) rats. Rats received either subcapsular implants or intraomental transplants, but never both. During the same surgery, some host

Key words: development, metanephros, transplantation, renal function, nephrectomy, chimeric kidneys, allograft, xenograft, organogenesis.

Received for publication November 21, 1997
and in revised form December 29, 1997
Accepted for publication January 5, 1998

rats had one kidney removed (UNX) or underwent unilateral nephrectomy and partial contralateral renal infarction (1½ NX) [11]. Others had both kidneys intact (NL).

Four metanephroi were implanted subcapsularly in each host kidney. Four to six weeks following transplantation, host kidneys containing metanephroi or metanephroi implanted in the omentum were removed from some host rats. When noted, kidneys were perfused prior to removal from host rats to remove blood from the organ [11].

In other UNX host rats that had received intraomental implants, end-to-end ureteroureterostomy was performed using a microvascular technique (interrupted 10-0 suture) between the ureter of a metanephros implanted in the omentum and the ureter of the kidney that had been removed. Four weeks later all remaining native renal tissue (the contralateral kidney) was removed from host rats, following which inulin and creatinine clearances were measured on conscious rats after placement of an indwelling bladder catheter and intravenous line exactly as in previous studies [12]. Baseline measurements for inulin were performed on urine and blood samples obtained prior to beginning the inulin infusions. These "background" values were subtracted from measurements performed after beginning the inulin infusion. Infusion of inulin was begun only following removal of all remaining native renal tissue and drainage of all urine remaining in the bladder (10 to 20 μl). Only the implanted metanephros remained connected to the bladder. As a control, we attempted to measure clearances in rats that had undergone bilateral nephrectomy, but had no transplanted metanephros connected to the bladder.

Kidneys were transplanted from one adult rat to another using techniques described in detail [13], except end-to-end anastomosis was performed between renal donor and recipient renal arteries instead of end-to-side anastomosis between the donor renal artery and recipient aorta. Briefly, donors were anaesthetized [11] and a midline incision was made to expose the left kidney. The donor was heparinized and the left kidney was flushed via the aorta with 5 ml of an ice-cold saline solution. Then, the kidney was removed *en block* after transecting the ureter near the bladder, the renal vein near the vena cava, and the renal artery near the aorta. The kidney was rinsed and transferred to a Petri dish containing ice-cold flush solution. The recipient was anesthetized, a midline incision made and the left kidney removed after transsecting the ureter near the hilum, the renal artery near its origin, and the renal vein near the kidney. End-to-end anastomoses were performed using 10-0 nylon suture, first between donor and recipient renal veins, then renal arteries, then ureters as described [13]. Following anastomoses, kidney were observed for a color change from dark-red to red so as to ascertain that blood flow was re-established.

Metanephroi or kidneys were fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin or with tetragonobolus purpurea lectin (TPL), exactly as in previous studies [14]. Dunnett's test was used for multiple comparisons [15]. All Figures are representative of at least five separate experiments.

RESULTS

As shown in Figure 1, metanephroi from E15 rat embryos were approximately 700 μm in diameter and had developing ureters (ureteric bud) attached (Fig. 1A). As shown previously [14], E15 metanephroi contained segments of ureteric bud that stained positively (red brown) with TPL (Fig. 1B, arrow) and condensing metanephric blastema (Fig. 1B, arrowhead), but contained no glomeruli.

Four or six weeks following renal subcapsular transplantation, when host kidneys were examined, cysts containing clear fluid surrounded the sites where metanephroi were transplanted under the capsule of NL, or 1½ NX rats. Two cysts under the capsule of a kidney from a NL rat four weeks post-transplant are shown in Figure 2A (arrowheads). Masses of tissue approximately 7 mm (7000 μm) in diameter were present under the cysts and were embedded into the larger host kidney. These structures were tenfold greater in diameter than transplanted metanephroi (approximately 1000-fold greater in volume). One such structure embedded into the parenchyma of a kidney from a 1½ NX rat four weeks post-transplant is shown in Figure 2B (arrowhead). Histological examination of hematoxylin and eosin-stained tissue revealed that the structures were integrated into the parenchyma of recipient kidneys, and that clusters of lymphocytes were present at the transplant-host kidney interface (Fig. 2C, arrowhead). Growth and developement took place in metanephroi transplanted into kidneys of either NL or 1½ NX rats that was indistinguishable. Metanephroi contained mature tubules (t) and glomeruli (g) that could be distinguished from tubule (T) and glomeruli (G) in adjacent recipient renal tissue by their smaller size, as in the studies of Abrahamson et al [5] (Fig. 2D). Figure 2E shows a glomerulus (g) and tubules (t) within a developed metanephros. No infiltration of lymphocytes into tubular or vascular structures of transplanted metanephroi was observed.

Levels of urea nitrogen and creatinine were measured in aspirated cyst fluid, and in blood from the aorta, and urine from the bladder of 1½ NX rats that had received metanephric implants. Levels of urea nitrogen were increased 2.6-fold and 15-fold, respectively, in cyst fluid and bladder urine relative to blood, and levels of creatinine were increased 12-fold and 28-fold, respectively (Table 1). Thus, both urea nitrogen and creatinine were concentrated in cyst fluid relative to blood. The concentrations of urea nitrogen and creatinine in cyst fluid were significantly less than the concentrations in bladder urine.

To learn more about how metanephroi became integrated into host kidneys, we examined kidneys of NL rats six weeks post-transplantation. To clear blood from the

Table 1. Measurements of creatinine and urea N in plasma, cyst fluid and bladder urine of 1½ nephrectomized rats that received subcapsular metanephric transplants ($N = 7$)

Plasma	Cyst fluid	Bladder urine
	creatinine/urea N *mg/dl*	
1.14 ± 0.08/53.8 ± 6.3	13.4 ± 2.2/136 ± 16	32.3 ± 5.2/800 ± 72

All measurements were made at the time of sacrifice. Comparisons were made using Dunnett's multiple comparison procedure [15]. For creatinine, plasma < cyst fluid, $P < 0.01$; and cyst fluid < bladder urine, $P < 0.01$. For Urea N (urea nitrogen), plasma < cyst fluid, $P < 0.01$; and cyst fluid < bladder urine, $P < 0.01$.

organ, kidneys were perfused using a modified Ringers solution injected into the aorta distal to the renal arteries following occlusion of the aorta proximal to the kidneys and transection of the inferior vena cava. This results in a blanching of the kidney as blood is replaced by perfusate. Normally, the entire kidney blanches [11]. However, following perfusion of kidneys that contained a transplanted metanephros, blood remained in the transplanted structure relative to the host kidney (Fig. 3A, arrows). The reason for the relative hypoperfusion of metanephric implants is unknown. It is possible that this reflects a more circuitous blood supply to the implant than to the native kidney, and therefore, one less prone to clearance by back-perfusion. Blood could be traced into the papilla of the host kidney (Fig. 3A, arrow). Histological examination of kidneys showed that glomeruli (g) in the transplanted kidney (Fig. 3B) had been poorly perfused relative to glomeruli present in the recipient kidney (G) (Fig. 3C), that is, the glomeruli in the transplants contained more red blood cells.

Kidneys were stained using hematoxylin and eosin or TPL [16], which binds to a carbohydrate moiety (α-L-Fucose) that is expressed in collecting ducts of developing rat kidneys prior to birth and for several weeks following birth [16], but not in collecting ducts of kidney from adult rats. In adult rat kidney the carbohydrate is expressed (and TPL binds) in distal tubules and medullary thick ascending limbs of Henle's loop [17]. Shown in Figure 4A is a hematoxylin and eosin-stained section of the interface between a perfused host kidney and a transplanted metanephros (arrows). Red blood cells are shown in blood vessels originating from the transplant (arrowhead). Figure 4B is the same section stained with TPL. An arrowhead shows red blood cells. The open arrow shows TPL-stained structures originating from the transplanted metanephros extending towards the papilla (P) of the host kidney. In adult host kidney tissue, TPL is expressed in cortex within distal tubule (DT) and medullary thick ascending limb (MTL), but not in collecting duct (open arrow) as would be expected (Fig. 4C). However, TPL is expressed in a population of collecting ducts (open arrow) that radiate from the transplanted metanephros into the papilla of the host kidney (Fig. 4D). These could represent collecting ducts derived from the implant. Alternatively, it is possible that host collecting tubules dedifferentiate in the presence of soluble factors secreted by embryonic grafts.

Metanephroi were next implanted in the omentum of six-week-old outbred Sprague-Dawley rats which then underwent unilateral nephrectomy. Six weeks later, the remaining renal tissue (native kidney and transplant) was removed from rats. When transplanted metanephroi (m) were examined post-transplantation, they had assumed a kidney-like shape *in situ* (Fig. 5A), and were approximately one-third the diameter of native kidneys (Fig. 5B). Transplanted metanephroi had intact ureters (u) (Fig. 5C). Sections of transplanted metanephroi were prepared and stained with hematoxylin and eosin. Both cortical and medullary tissue was present (Fig. 5D). Cortices contained well-developed glomeruli containing red blood cells (g), proximal tubules (p) with well-developed brush border membranes (arrowhead), and distal tubules (d) (Fig. 5E). Medullas contained well-developed collecting ducts (cd) (Fig. 5F). Rare accumulations of lymphocytes were observed (not shown), but there was no lymphocytic infiltration of tubular or vascular elements.

In contrast to findings in rats that underwent unilateral nephrectomy at the time of implantation, little or no growth of metanephroi occurred when they were implanted in the omentum of rats that had no native renal tissue removed (not shown).

In seven rats that had received transplanted metanephroi six weeks previously in which unilateral nephrectomy also had been performed, the ureter of a transplanted metanephros was anastomosed to the ureter of the kidney that was removed. Four weeks later, the contralateral kidney was removed, following which inulin was infused into the rat and clearances measured. Plasma creatinines at the time of measurements (following removal of all native renal tissue) were 1.3 ± 0.06 mg/dl, approximately three times the normal values. Inulin and creatinine clearances were 0.11 ± 0.02 and 0.65 ± 0.18 μl/min/100 g body wt, respectively (mean ± SE). The mean body wt of rats was 238 ± 3.0 g. The mean weight of metanephroi was 71 ± 15 mg. The mean volume of urine collected during three hours was 49 ± 13 μl. Inulin and creatinine clearances in a group of five normal rats of similar size were 0.92 ± 0.14 and 0.84 ± 0.12 ml/min/100 g, respectively (Table 2).

As a control, we attempted to measure clearances in three rats that had undergone bilateral nephrectomy, but had no transplanted metanephros connected to the bladder. However, in contrast to the case in rats with a transplanted metanephros connected to the bladder, no urine appeared in the bladder catheter over a three-hour collection time in rats that had no transplanted metanephros.

Shown in Figure 6 is a ureteroureterostomy between the ureter of metanephros transplanted in the omentum and the host rat ureter (arrow) performed six weeks after metanephros implantation (Fig. 6A), an artery originating

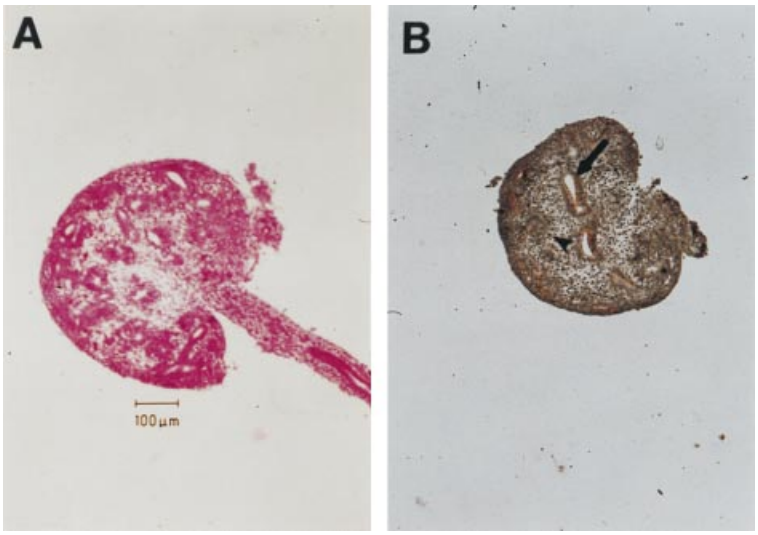

Fig. 1. An embryonic day 15 (E15) rat metanephros stained with hematoxylin and eosin (*A*) or tetragonobolus purpurea lectin (TPL), counterstained with hematoxylin (*B*). Arrow shows the ureteric bud branch, and the arrowhead shows a non-staining developing nephron. Magnification is shown.

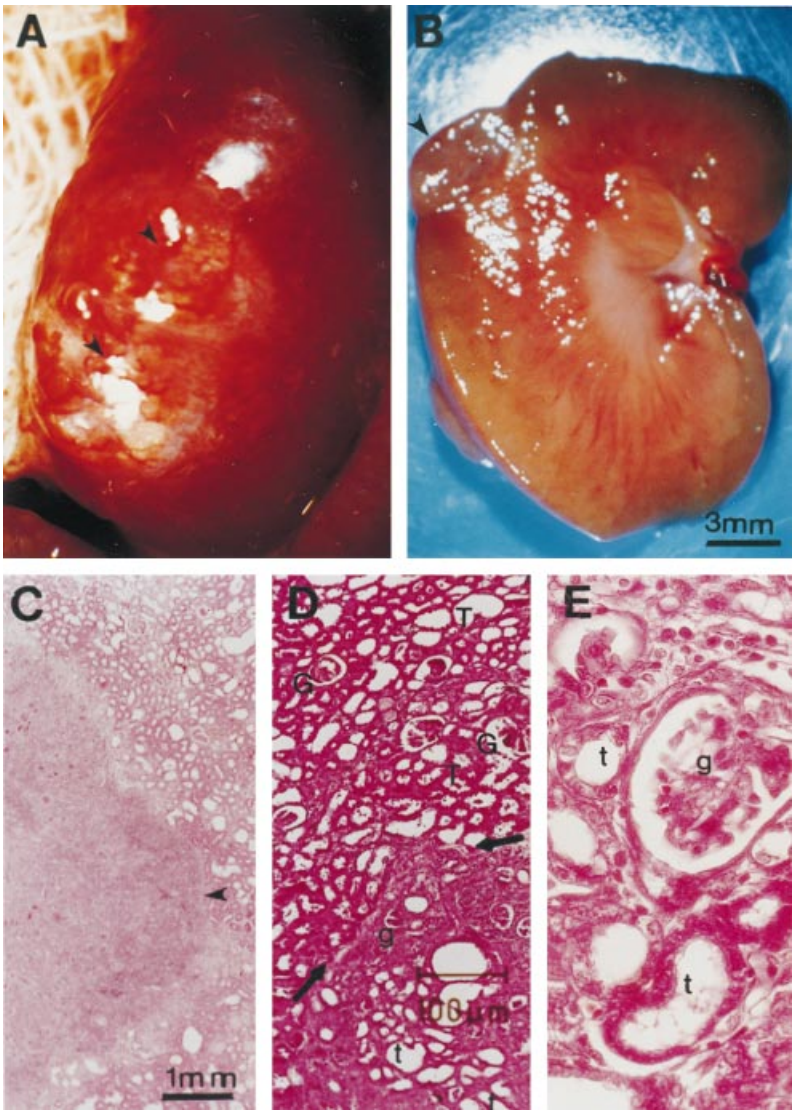

Fig. 2. (*A*) Cysts (arrowheads) in a whole kidney from a normal (NL) rat four weeks post-transplantation of metanephroi. (*B*) A mid-sagittal section of a kidney from a partial contralateral renal infarction (1½ NX) in a rat four weeks post-transplantation of metanephroi showing structure within a cyst (arrowhead) embedded in the parenchyma. (*C*) Hematoxylin and eosin-stained mid-sagittal section of a kidney from a 1½ NX rat four weeks post-transplantation of metanephroi showing incorporation of a metanephros into the parenchyma of the host kidney and lymphocytic infiltrate (arrowhead). (*D*) Interface (arrows) between transplanted metanephros (bottom) containing glomeruli (g) and tubules (t) and host kidney of a 1½ NX rat (top) containing larger glomeruli (G) and tubules (T). (*E*) Metanephros transplanted into a kidney of a NL rat containing a glomerulus (g) and tubules (t). Magnifications are shown.

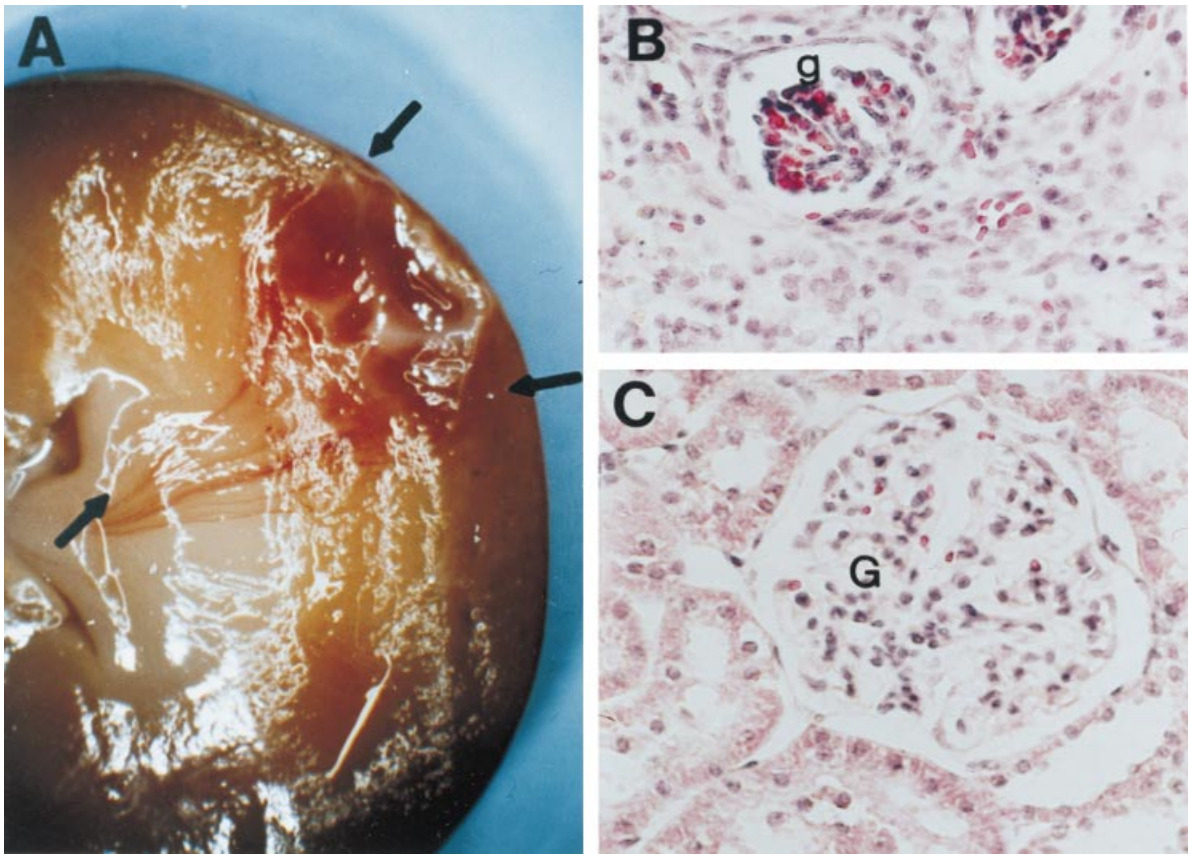

Fig. 3. A mid-sagittal section obtained following perfusion of a kidney originating from a normal (NL) rat six weeks post-transplantation. (*A*) Arrows outline the relatively poorly-perfused transplant. (*B and C*) Photomicrographs of hematoxylin and eosin-stained kidneys where (B) shows the glomerulus (g) within the transplanted kidney and (C) illustrates the glomerulus (G) within host kidney.

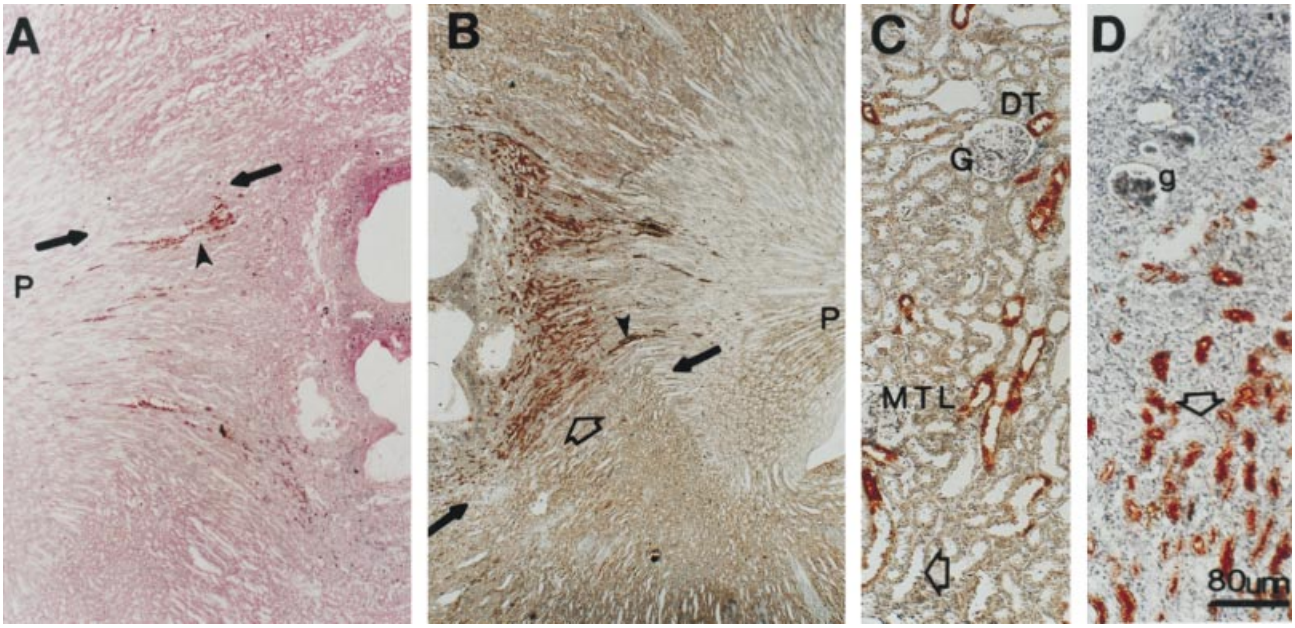

Fig. 4. Hematoxylin and eosin (*A*) or tetragonobolus purpura lectin (TPL)-stained (*B through D*) sections of a transplanted metanephros and a kidney from a normal (NL) host rat (A and B). Arrows show the transplant-host interface. Arrowheads show red blood cells in vascular structure. (B-D) Open arrows show the collecting ducts. (C) Shown is a glomerulus (G), distal tubules (DT) and medullary thick ascending limbs of Henle's loop (MTL) within host kidney and collecting ducts (open arrow). (B and D) Shown are collecting ducts (open arrows) radiating inward towards the host papilla (P) from the transplanted kidney. A glomerulus in the transplant (g) is labeled. Magnification is shown for C and D.

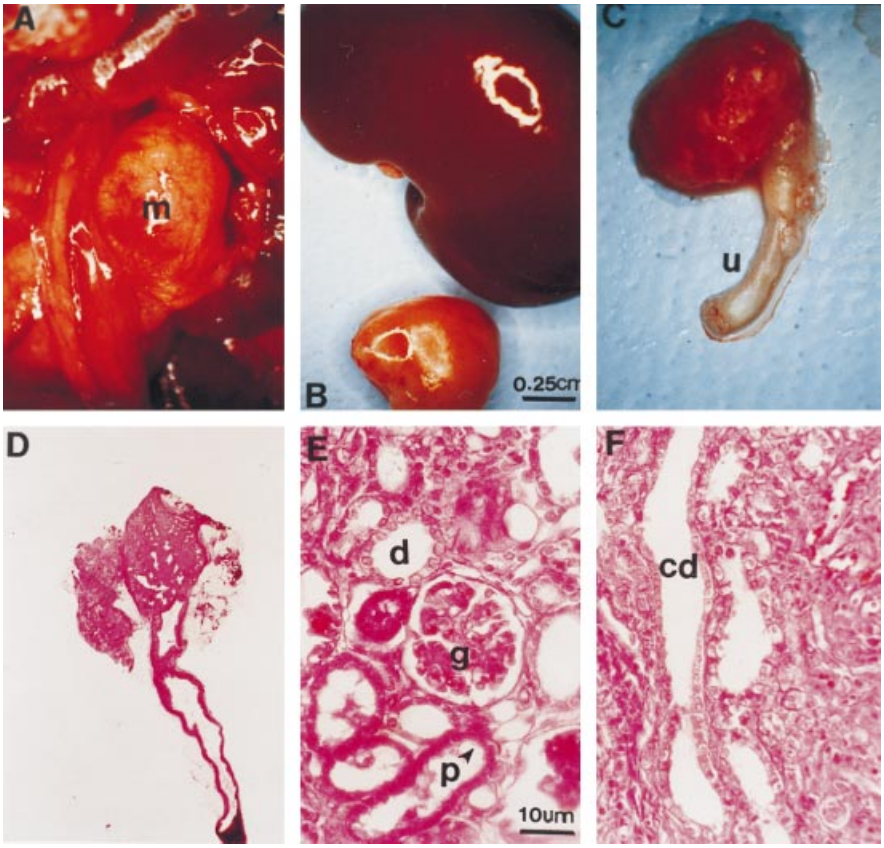

Fig. 5. Rat metanephroi, six weeks post-transplantation into the omentum of unilaterally nephrectomized host rats and photomicrographs of hematoxylin and eosin-stained sections of metanephroi. (*A*) Developed metanephros in abdominal cavity (m). (*B*) Developed metanephros after removal from abdominal cavity compared to native kidney. (*C*) Developed metanephros. The ureter (u) is shown. (*D*) Section of developed metanephros with ureter. (*E*) Section of cortex from developed metanephros. The glomerulus (g), proximal tubule (p), brush border (arrowhead) and distal tubule (d) are shown. (*E*) Section of medulla from developed metanephros. The collecting duct (cd) is shown. Magnifications for A-D are shown in (B), and for E and F in (E).

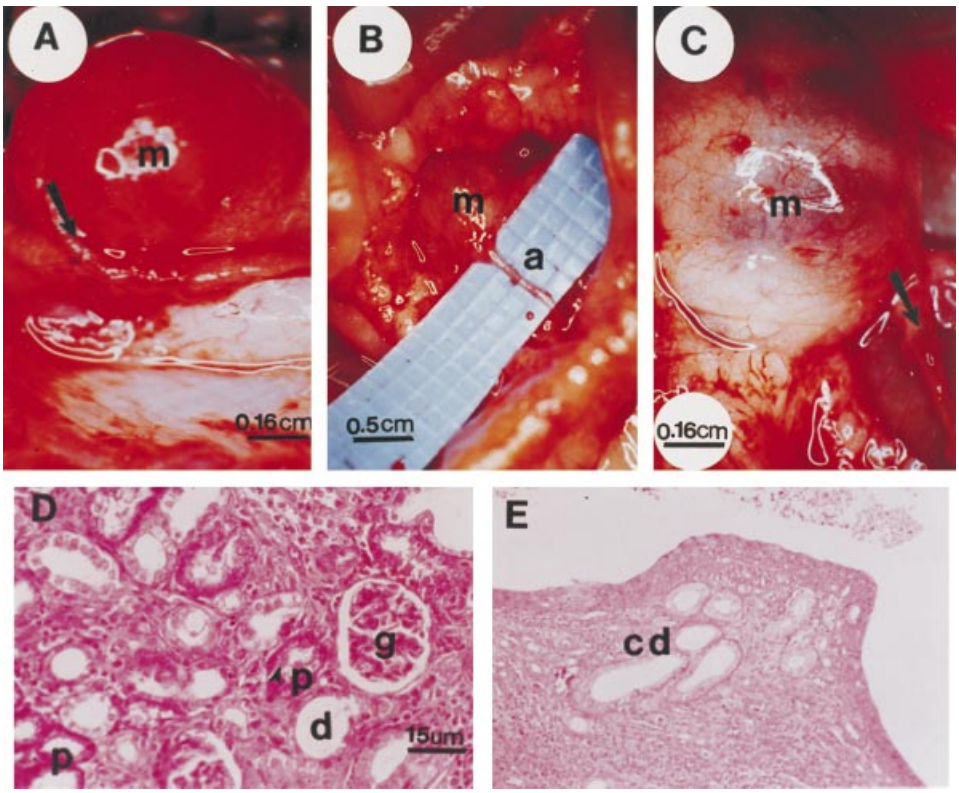

Fig. 6. Rat ureters (*A*) or a metanephros implanted in a unilaterally nephrectomized rat (*B and C*), six weeks (A) or 10 weeks (B, C) post-transplantation into the omentum of host rats and photomicrographs of hematoxylin and eosin-stained sections of a metanephros 10 weeks post-transplantation (*D and E*). Anastomosis is shown (arrow in A and C) between host ureter and ureter from implanted metanephros (m). An artery originating from the omentum (a) is shown (C); Glomerulus (g), proximal tubule (p), brush border (arrowhead), distal tubule (d) and collecting duct (cd) are shown (D and E). Magnifications are shown for A-C, and for D-E.

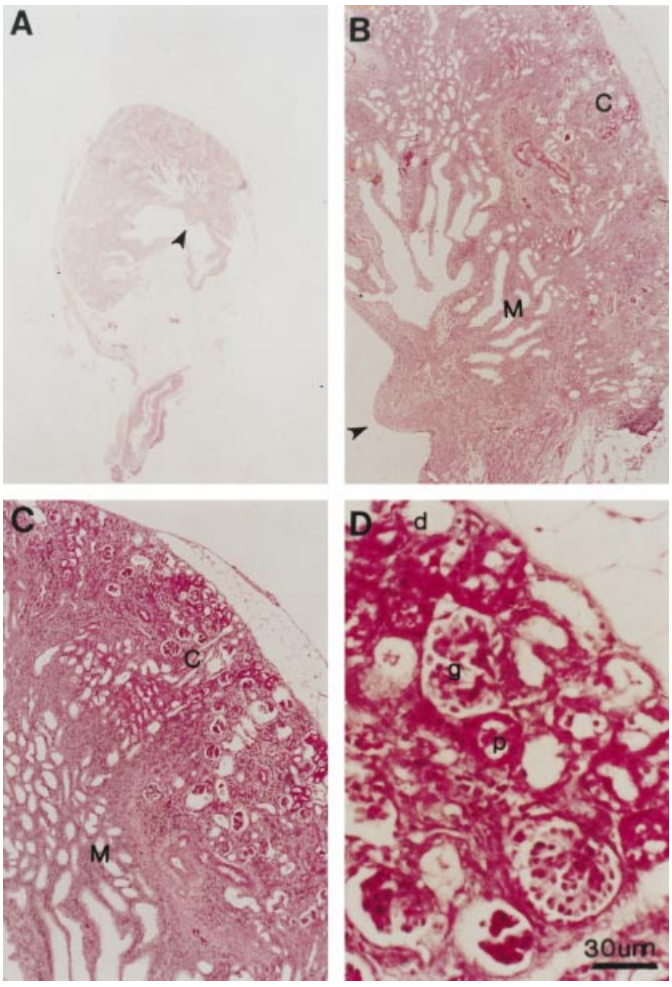

Fig. 7. Progressively enlarged photomicrographs (A→D) of a single metanephros 10 weeks post-transplant into a unilaterally-nephrectomized host, stained with hematoxylin and eosin. A renal papilla is shown in panels *A and B* (arrowheads). Cortex and medulla (C and M) are labeled in *B and C*. A glomerulus (g), proximal tubule (p) and distal tubule (d) are labeled in *D*. Magnification is shown in panel D.

Fig. 8. Progressively enlarged photomicrographs (A→B) of a single metanephros 10 weeks post-transplant into a host that underwent unilateral nephrectomy and partial contralateral renal infarction, stained with hematoxylin and eosin. Cortex and medulla (C and M) are labeled in panel *A*. A glomerulus (g), and proximal tubule (p) are labeled in *B*. Magnification is shown in panel B.

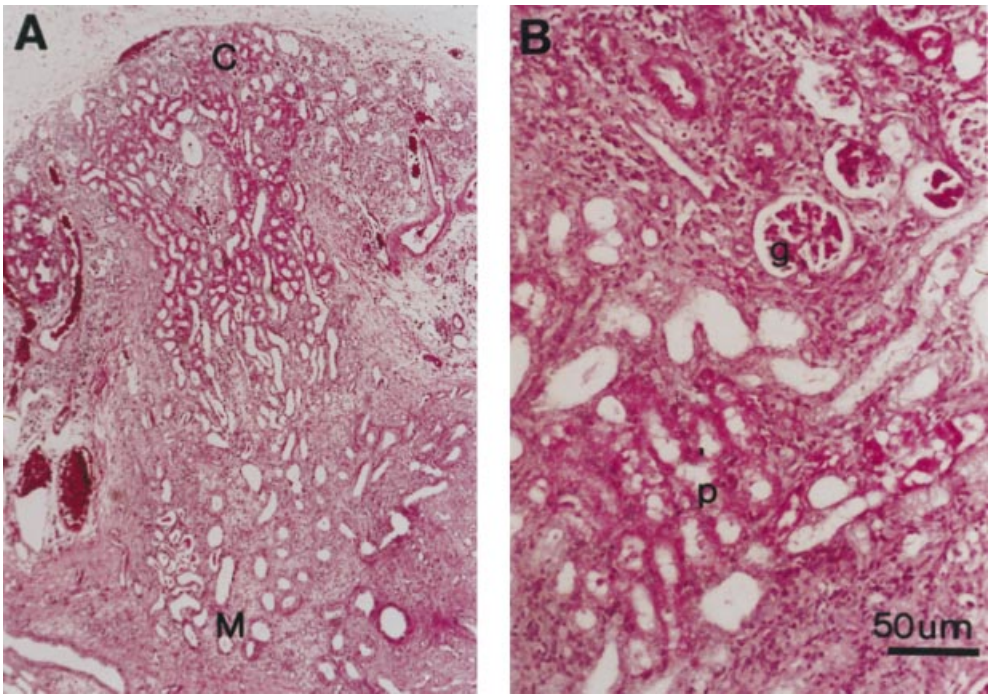

Table 2. Values measured in normal rats and in nephrectomized host rats with an omental metanephric implant (Imp)

	Imp/UNX ($N = 7$)	Imp/UNX+ ($N = 3$)	Normal (2 kidneys) ($N = 5$)
Creatinine *mg/dl*	1.3 ± 0.06	1.2 ± 0.05	0.5 ± 0.02
Renal mass *g*	0.07 ± 0.02	0.15 ± 0.02[a]	3.16 ± 0.06
Inulin clearance			
μl/min/100 g	0.11 ± 0.02	0.97 ± 0.33[b]	920 ± 140
μl/min/g kidney wt	2.42 ± 0.70	30.1 ± 8.7[b]	750
Creatinine clearance			
μl/min/100 g	0.65 ± 0.18	1.2 ± 0.4	840 ± 120
μl/min/g kidney wt	26.7 ± 6.9	31.6 ± 4.5	710

KW, kidney weight; UNX, unilateral nephrectomy; UNX+, unilateral nephrectomy plus partial contralateral renal infarction. Data are mean ± SE.

Implant/UNX vs. Implant/UNX+: [a] $P < 0.05$; [b] $P < 0.01$, Student's *t*-test

from the omentum (a) supplying a metanephros (m) that had been implanted 10 weeks previously (Fig. 6B), an intact ureteroureterostomy (arrow) four weeks after the anastomosis was performed (Fig. 6C), and hematoxylin and eosin-stained sections of metanephroi implanted 10 weeks previously showing glomeruli (g), proximal tubules (p), distal tubules (d) (Fig. 6D) and collecting duct (cd) (Fig. 6E).

Shown in Figure 7 are four progressively enlarged views (A→D) of one of the developed metanephroi used to generate the data shown in Table 2. A renal papilla is shown (Fig. 7 A, B, arrowheads). Cortex and medulla (C and M) are labeled (Fig. 7 B, C). A glomerulus (g), proximal tubule (p) and distal tubule (d) are labeled (Fig. 7D).

Figure 8 shows sections of a metanephros 10 weeks following implantation into the omentum of a rat that underwent unilateral nephrectomy and partial contralateral infarction at the time of implantation. Weights of transplanted metanephroi were increased more than twofold and inulin clearances expressed per g of kidney weight were increased more than 12-fold compared to values obtained in rats that underwent unilateral nephrectomy without partial contralateral renal infarction (Table 2). Urine volumes (145 ± 24 μl/3 hr) were also significantly increased ($P < 0.005$, Student's *t*-test).

Transplantation of developed kidneys from one Sprague-Dawley rat to another is possible if the hosts are treated with cyclosporine A. On the other hand, without immunosuppression, the renal architecture is obliterated by an intense mononuclear cell infiltrate with destruction of the tubules by seven days post-transplantation [13]. To ascertain whether a similar rejection would occur in the population of Sprague-Dawley rats used in our studies, we transplanted kidneys from one adult rat to another. At seven days post-transplantation, the graft was swollen and the surface somewhat mottled in appearance (Fig. 9A) compared to the contralateral host kidney (Fig. 9B). As in previous studies [13], the renal architecture was obliterated

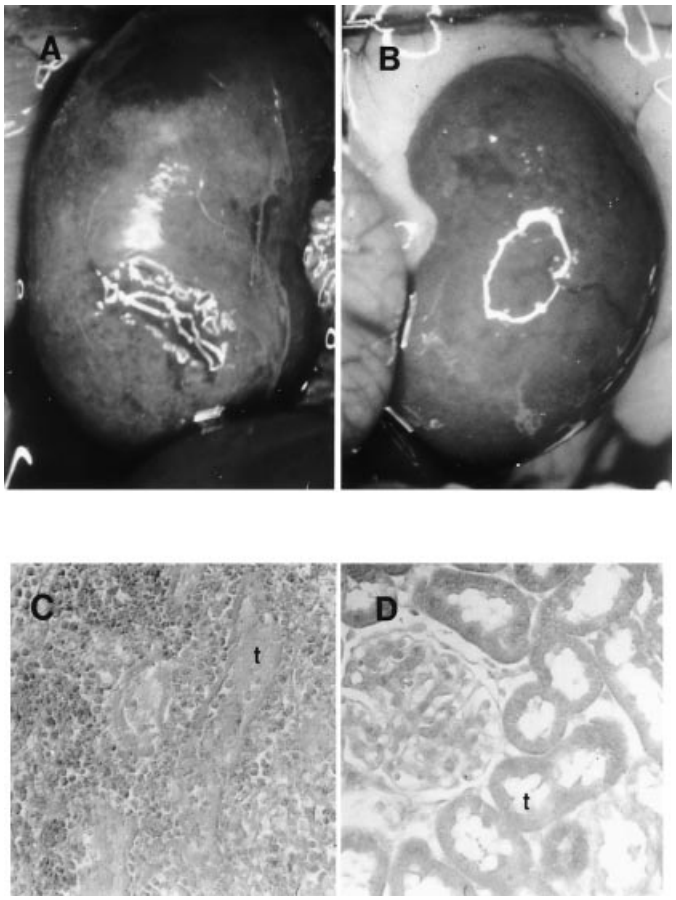

Fig. 9. Photographs (*A and B*) and photomicrographs (*C and D*) of a kidney seven days post-transplantation from one adult Sprague-Dawley rat to another Sprague-Dawley rat (A, C) and the contralateral native kidney (B, D). Sections shown in C and D are stained with hematoxylin and eosin. Proximal tubules are shown (t).

by an intense mononuclear cell infiltrate with destruction of the tubules by seven days post-transplantation (Fig. 9C). The contralateral kidney had a normal architecture as shown in Figure 9D.

DISCUSSION

The metanephric kidneys originate during the fifth week of gestation in humans, and during day 12 of embryonic rat development (E12) when outgrowths of the mesonephric ducts, so-called ureteric buds, collect about their distal ends, intermediate mesoderm caudal to the mesonephros, designated metanephric blastema. Numerous outgrowths arise from the distal end of the ureteric bud that push radially into the surrounding mass of metanephric blastema and give rise to the collecting ducts of the kidneys. The proximal ends of the ureteric bud give rise to the ureter and renal pelvis. The metanephric blastema differentiates into all of the tubular structures of the adult nephron with the exception of the collecting system, which originates from the ureteric bud [3]. Humans develop a full complement of nephrons by approximately 35 weeks of gestation [18].

However, in rodents nephrogenesis is not complete at the time of birth, but rather continues for the first two to three weeks following birth. Nephrons continue to develop from a nephrogenic zone located at the periphery of the kidney [19].

Once renal development is complete, no new nephrons are formed under any conditions. The loss of renal functional mass that occurs following insults to the adult kidney is compensated for in the short term, by hypertrophy and hyperfunction of the remaining nephrons [20]. However, these compensatory changes are often transient and under some circumstances maladaptive in that they may lead to further loss of renal function [21].

The transplantation of developing renal tissue into adult animals has been accomplished in a number of studies. Several sites, including the anterior eye chamber, have been shown to support kidney tubule development and vascularization of the transplant. In addition, the possibility that renal function can be enhanced through addition of functioning nephrons via transplantation of allograft metanephroi intrarenally or intraabdominally has been explored.

Woolf et al implanted pieces of sectioned metanephroi originating from embryonic day (E)13 to E16 mice into tunnels fashioned in the cortex of kidneys of newborn outbred mice. Differentiation and growth of donor nephrons occurred in the host kidney. Glomeruli were vascularized, mature proximal tubules were formed and extensions of metanephric tubules into the renal medulla were observed. Glomerular filtration was demonstrable in donor nephrons using fluorescently-labeled dextran as a marker of filtration into the proximal tubules. In contrast to the case in newborn mice, metanephric tissue transplanted into kidneys of adult mice neither grew nor differentiated, but was extruded as a mass under the renal capsule resembling a poorly-differentiated tumor. It was concluded that the neonatal kidney, which has a rim of undifferentiated cortex (the nephrogenic zone), can facilitate the differentiation of an embryonic implant, but that this ability is lacking in the fully-differentiated adult kidney [7].

Abrahamson et al implanted metanephroi from E17 rat embryos beneath the renal capsule of five adult rat hosts. Within 9 to 10 days post-implantation, every graft became vascularized, new nephrons were induced to form and glomerular and tubular cytodifferentiation occurred. Glomeruli from transplanted metanephroi were identifiable because they were approximately two-thirds the diameter of those within host kidneys. Intravenous injection of antilaminin IgG into hosts resulted in labeling of glomerular basement membranes of grafted kidneys, confirming perfusion of the grafts by the host's vasculature. Four hosts were sacrificed at 10 days post-transplantation and one at 28 days. Signs of rejection such as hypercellular glomeruli and lymphocytic infiltrates in peritubular spaces were obvious by 10 days post-transplantation [5].

Robert et al grafted metanephroi from E12 mouse embryos into kidney cortices of adult and newborn ROSA26 mouse hosts. ROSA26 mice bear an ubiquitously expressed β-galactosidase transgene that can be identified by staining in histological sections, permitting differentiation of transplanted from host tissue. Grafts into both newborn and adult hosts examined seven days post-transplantation were vascularized by components originating from both donor and host [6].

Barakat and Harrison sectioned metanephroi originating from E15-E17 rat embryos into four quarters and each quarter was transplanted into a subcutaneous site in the abdominal wall of closely related or unrelated male or female adult rats. The vascularity originally present in the E15-E17 metanephroi remained intact for two to three days following transplantation. Grafting sections of metanephroi into unrelated adults resulted in lymphocytic infiltration of the graft by 8 to 9 days post-transplantation and replacement of the graft by fibrosis by the 11 to 12th day. Grafting sections into related adults (mothers or fathers of donor offspring taken from a closed colony following 15 generations of brother to sister matings) resulted in lymphocytic infiltration of the graft by 12 to 19 days post-transplantation and replacement of the graft by fibrosis by the 14 to 22nd day [8].

Koseki et al transplanted rat nephrogenic mesenchymal cells that had been transfected with a Lac Z reporter gene by a retrovirus, underneath the capsule of kidneys of neonatal rats. Transplanted mesenchymal cells were integrated into functioning host nephron segments [9].

To our knowledge, our studies show the first long-term survival (greater than 10 days) of metanephroi transplanted subcapsularly into fully differentiated kidneys of animals in which nephron formation is no longer taking place, and the first intraomental transplantation of metanephroi. The observations are of interest from three perspectives.

First, neither the renal subcapsular space nor the omentum is known to be an immunologically privileged site [22]. It is not possible to transplant developed kidneys from one adult outbred Sprague-Dawley rat to another without using immunosuppression (Fig. 9) [13]. Our findings suggest that the immune response to transplanted metanephroi may be muted relative to the response to transplanted developed kidneys.

Neither Wolff et al [7] nor Abrahamson et al [5] were able to transplant rodent metanephroi subcapsularly into kidneys of adult hosts and have them survive without rejection for longer than 10 days [5]. The difference between their findings and ours may be explained by a difference in technique. Wolff et al transplanted pieces of sliced metanephroi [7], whereas we transplanted whole metanephroi with capsules intact. Antigens present within the intact metanephroi would be expected to have reduced exposure to host antibodies or cells mediating rejection. Abrahamson et al transplanted metanephroi from E17 rats

[5], whereas we used E15 rats. It was noted that the E17 metanephroi were more immunogenic than the E15 metanephroi when they were transplanted into the anterior chamber of the eye, possibly because of the acquisition of additional tissue antigens by E17 [5].

There are several possible explanations for the reduced immunogenicity of transplanted metanephroi. (*a*) The transplanted metanephros becomes a chimeric organ [5–7] in that it is vascularized in part by blood vessels originating from the host kidney. Rejection on the basis of antigens present on the vascular endothelium of the transplant would be reduced in severity if the transplanted organ were supplied by host vessels. (*b*) The relatively undeveloped, avascular E15 metanephros is likely to contain fewer antigen presenting cells than a developed kidney, rendering it less immunogenic [4]. (*c*) The absence of one or more co-stimulatory molecules such as B7 could dampen the immune response. In mouse kidney, the lack of B7 expression in proximal tubule cells restricts their ability to stimulate $CD4^+$ cells *in vitro* [23].

Second, our data and those of Abrahamson et al [5] indicate that ongoing nephrogenesis, such as that present in the nephrogenic zone of kidneys in newborn rodents, is not an absolute requirement for engraftment of transplanted metanephroi. Not only can metanephroi be transplanted into a fully-differentiated adult kidney, but new glomeruli and tubules form in the implant. In this manner, it is possible to add new (transplanted) nephrons to differentiated adult kidney. Given this possibility, it must be considered whether these new nephrons function (effect clearance from plasma) or can be induced to function in hosts.

Woolf et al described the presence of glomeruli, proximal tubules and linear structures of donor origin resembling loops of Henle, originating from the region of developed metanephroi implanted into kidneys of newborn hosts. These latter structures extended into the medullary regions of the host [7]. We have shown that glomeruli and tubules develop within metanephroi transplanted into kidneys of adult rats (Fig. 2). In addition, structures with the staining characteristics of collecting ducts, extent from metanephroi towards the papilla of the host (Fig. 4). However, neither Woolf et al [7] nor we provide evidence that metanephroi transplanted subcapsularly function in a manner similar to native kidneys or effect clearance from plasma of hosts. Such a function would require that tubular components of nephrons from transplants develop in an anatomically correct manner and clearance would require that they connect with the host's collecting system. Definitive evidence of such anatomical correctness and of such connections can probably only be obtained by careful microdissection, which was not performed by Woolf et al [7] or in our studies.

Third, the findings of Woolf et al are consistent with the presence of glomerular filtration within developed mouse metanephroi transplanted subcapsularly into kidneys of newborn hosts [7]. Our observation that concentrations of BUN and creatinine increase significantly between serum and cyst fluid and again between cyst fluid and bladder urine (Table 1) raises the possibility that the cyst fluid represents urine originating from the transplant. That cyst fluid is dilute relative to bladder urine is consistent with the reduced ability of a four-week-old kidney (transplanted kidney) to clear the blood of urea nitrogen and creatinine relative to a 10-week-old kidney (host kidney) [24].

Glomerular filtration in metanephroi transplanted subcapsularly in kidneys was demonstrated by Woolf et al [7] and by Abrahamson et al [5]. However, our studies using metanephroi transplanted into the omentum are the first to demonstrate plasma clearance in this setting. Creatinine clearances were considerably higher than inulin clearances consistent with tubular secretion of creatinine at very low rates of glomerular filtration [25]. Inulin clearances expressed per 100 g body wt were very low compared to normal in Sprague-Dawley rats of approximately the same size. However, kidneys are capable of increasing glomerular filtration considerably, even after all nephrons are formed. For example, single nephron glomerular filtration rate in rats increases approximately 18-fold between 22 to 60 days of life [24]. Therefore, it is possible that over time, the rates of glomerular filtration of transplanted metanephroi could increase sufficiently so as to provide a level of renal function high enough to sustain life.

Data shown in Table 2 demonstrate an increase of inulin clearance of more than 12-fold in rats that underwent unilateral nephrectomy and partial contralateral renal infarction compared to those that underwent only unilateral nephrectomy at the time of implantation. Ten weeks following the further reduction of renal mass, transplanted metanephroi were more than twice as heavy (145 vs. 71 mg). These observations, coupled with our finding that growth and development of transplanted metanephroi do not occur if no native renal mass is removed at the time of implantation, indicate that the stimulus that results in compensatory renal growth following reduction of renal mass [26] may also enhance the growth and development of transplanted metanephroi. The identity of the stimulus remains unknown. Although changes in the expressions of several growth factors in kidney accompany compensatory renal growth, no growth factor has been identified as causative of this process [26].

ACKNOWLEDGMENTS

S.A.R, N.A.H. and M.R.H. were supported by grants DK-27600, DK-20579 and DK-07126 from the National Institutes of Health. We acknowledge useful discussions with Drs. Paul Lacy and John Kissane (Washington University).

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APPENDIX

Abbreviations used in this article are: D, distal tubule; E, embryonic day; MTL, medullary thick ascending limb; NL, normal; 1½ NX, unilateral nephrectomy and partial contralateral renal infarction; P, papilla; TPL, tetragonobolus purpurea lectin; UNX, unilateral nephrectomy.

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